

REMARKS

The April 16, 2004 Office Action indicates that claims 102, 103, 108, 109 and 111-124 are pending in the subject application. However, applicants understand this to be a clerical error, and that the outstanding Office Action should indicate that claims 101, 102, 108, 109 and 111-124 are pending in the subject application. In applicants' December Amendment, applicants, in relevant part, canceled claim 103, and amended claim 101.

Applicants hereinabove have amended the specification, cancelled claim 102 and amended claims 101, 108, 109, 111-114, 117, 123 and 124. Accordingly, upon entry of this Amendment, claims 101, 108, 109 and 111-124, as amended, will be pending and under examination.

Applicants maintain that the amendments to the specification and to claims 101, 108, 109, 111-114, 123 and 124 do not raise any issue of new matter, and that these claims, as amended, are fully supported by the specification as originally filed.

Applicants have amended claim 117 to correct a minor typographical error. Support for the claim amendments is found, *inter alia*, in the specification as follows: **Claims 101 and 111-114**: page 11, line 33 to page 12, line 29, page 12, lines 15-16, page 14, lines 1-5, page 32, lines 13-20, and Figures 1-1 and 1-2; **Claim 108**: page 87, lines 27-29, and page 37, lines 31-35; **Claims 109, 123 and 124**: page 12, lines 15 and 16, page 16, lines 26-28, and page 66, lines 8-14.

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 15

In view of the comments set forth below, applicants maintain that the grounds of the Examiner's rejections made in the April 16, 2004 Office Action have been overcome, and respectfully request that the Examiner reconsider and withdraw these grounds of rejection.

February 3, 2004 Examiner's Proposed Amendment, February 6, 2004 Examiner's Interview and August 12, 2004 Examiner's Interview

On February 3, 2004, the Examiner assigned to related copending applications (U.S. Serial No. 08/196,154, 08/477,097 and 08/481,809) forwarded to the undersigned's office proposed Examiner's Amendments for such related copending applications which applicants understood would place such applications in condition for allowance. For completeness of the record, applicants submit herewith as **EXHIBITS A-C** copies of the February 3, 2004 Examiner's Proposed Amendments in connection with related U.S. Serial No. 08/196,154, 08/477,097 and 08/481,809, respectively.

On February 6, 2004, applicants' undersigned attorney, Mark A. Farley, Esq., had a telephonic interview with Examiner Holleran concerning the February 3, 2004 Examiner's Proposed Amendment in connection with related U.S. Serial No. 08/196,154, 08/477,097 and 08/481,809. For completeness of the record, applicants submit herewith as **EXHIBITS D-F** copies of the Interview Summaries for those interviews conducted in connection with related U.S. Serial No. 08/196,154, 08/477,097 and 08/481,809, respectively.

Subsequently, on April 16, 2004, Examiner Holleran issued the Office Action to which this Amendment is a response.

Thereafter, on August 12, 2004, the undersigned had a telephonic interview with Examiner Holleran concerning the February 3, 2004 Examiner's Proposed Amendment in the related U.S. application (U.S. Serial No. 08/196,154), which is similar to the February 3, 2004 Examiner's Proposed Amendment in the subject application. For completeness of the record, applicants submit herewith as **EXHIBIT G** a copy of the Interview Summary for that interview.

Applicants have carefully reviewed the February 3, 2004 proposed Examiner's Amendments for related copending applications (U.S. Serial No. 08/196,154, 08/477,097 and 08/481,809) and have substantially incorporated it into this Amendment. However, applicants have made certain changes which are believed necessary, for example, to correct minor typographical errors and to insure proper antecedent basis in the amended claims. Applicants maintain that this Amendment places this application in condition for allowance and look forward to receiving from the Examiner a communication to this effect.

Rejections Withdrawn In April 16, 2004 Office Action

The Examiner stated that the objection to the disclosure is withdrawn in view of the submission of a new "Figure 6B."

The Examiner stated that the provisional rejection of

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 17

claims 101-125 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 123-146 of copending Serial No. 08/477,147 is withdrawn in view of the terminal disclaimer filed December 15, 2003.

The Examiner also stated that the rejection of claims 104-106 and 125 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement, is withdrawn in view of the cancellation of the claims. The Examiner also stated that for clarification of the record, it is noted that in the Amendment filed December 15, 2003 (which applicants interpret to mean applicants' December 10, 2003 Amendment), applicants indicated an amendment to claim 124 and then a second "claim 124". that was cancelled. The Examiner also stated that the second "claim 124" is assumed to be a typographical error, and is interpreted to mean that claim 125 was cancelled.

The Examiner also stated that the rejection of claims 101-111 under 35 U.S.C. §103(a) as being unpatentable over Wiegand et al. (U.S. Patent 5,599,914, issued February 4, 1997) in view of Fiume et al. (Critical Rev. Therapeutic Drug Carrier Systems, 4(4):265-284 (1988)), Ritter et al. (Seminars in Cancer Biology, 2:401-409 (1991)), Kensil et al. (The Journal of Immunology, 146(2):431-437 (1991)), Marciani et al. (Vaccine, 9:89-96 (1991)) and Uemura et al. (J. Biochem., 79(6):1253-1261 (1976)) is withdrawn.

The Examiner also stated that the rejection of claims

101, 111-114 and 116-125 under 35 U.S.C. §103(a) as being unpatentable over Wiegand et al. (U.S. Patent 5,599,914, issued February 4, 1997), Fiume et al. (Critical Rev. Therapeutic Drug Carrier Systems, 4(4):265-284 (1988)), Livingston et al. (Cancer Research, 149:7045-7050 (1989)), in view of Ritter et al. (Seminars in Cancer Biology, 2:401-409 (1991)), Livingston et al. (U.S. Patent No. 5,102,663), Kensil et al. (The Journal of Immunology, 146(2):431-437 (1991), Marciani et al. (Vaccine, 9:89-96 (1991)) and Uemura et al. (J. Biochem., 79(6):1253-1261 (1976)) is withdrawn.

The Examiner further stated that the rejection of claims 114 and 115 under 35 U.S.C. §103(a) as being unpatentable over Wiegand et al. (U.S. Patent 5,599,914, issued February 4, 1997) in view of Fiume et al. (Critical Rev. Therapeutic Drug Carrier Systems, 4(4):265-284 (1988)), Livingston et al. (Cancer Research, 149:7045-7050 (1989)), Ritter et al. (Seminars in Cancer Biology, 2:401-409 (1991)), Livingston et al. (U.S. Patent No. 5,102,663), Kensil et al. (The Journal of Immunology, 146(2):431-437 (1991), Marciani et al. (Vaccine, 9:89-96 (1991)) and Uemura et al. (J. Biochem., 79(6):1253-1261 (1976)) as applied to claims 78, 80-92, 94 and 96-99 above and further in view of Diatlovitskaia et al. (U.S. Patent No. 4,557,931) is withdrawn.

Formalities

The Examiner objected to claims 101 and 111 because these claims do not end in a "period" and therefore are not complete sentences. The Examiner also stated these

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 19

claims appear to be incomplete because they end with the word "and."

In response to Examiner's objections to claims 101 and 111, applicants have amended these claims to address the Examiner's objections.

Terminal Disclaimer filed December 15, 2003

As mentioned above, applicants filed a Terminal Disclaimer on December 10, 2003 (which the U.S. Patent and Trademark Office received on December 15, 2003) relative to any patent issuing from copending U.S. Serial No. 08/477,147 in response to then outstanding provisional obviousness-type double patenting rejections of claims 101-125.

Applicants intend to submit a substitute Terminal Disclaimer with respect to any patent issuing from any one or more of copending U.S. Serial Nos. 08/196,154, 08/477,097, 08/477,147, and/or 08/481,809 in the near future. Applicants note that the substitute Terminal Disclaimer will replace and supersede in all respects the Terminal Disclaimer filed December 15, 2003.

Claim Rejection Under 35 U.S.C. §112, First Paragraph - Written Description

The Examiner rejected claims 101, 102, 108, 109 and 111-124 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at

the time the application was filed, had possession of the claimed invention. Specifically, the Examiner stated that the specification does not support the genus of conjugates comprising a GD3 lactone ganglioside having an "altered ceramide portion comprising an altered sphingosine base."

The Examiner stated that the claimed inventions read on compositions comprising GD3 lactone ganglioside conjugates and methods of treatment comprising the administration of compositions comprising GD3 lactone ganglioside conjugates, where the ganglioside portions of the conjugates are so broadly claimed that they are not adequately described by the specification. Specifically, the Examiner stated that the recitation "GD3 lactone ganglioside derivative" that comprises "an altered ceramide portion comprising an altered sphingosine base" refers to a genus of compounds that is not supported by the specification. The Examiner stated that the only example of an "altered ceramide portion comprising an altered sphingosine base" provided by the specification is the one example of a ganglioside conjugate in which, prior to conjugation, the sphingosine base has been cleaved with ozone and reduced to form a reactive aldehyde at the C-4 carbon of the sphingosine base. The Examiner also stated that this one example is not representative of all the possible species encompassed by the phrase "ganglioside derivative which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base." The Examiner thus concluded that the genus of conjugates is not supported by an adequate

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 21

written description of the varied members of the genus, and one of skill in the art would not find that applicants were in possession of the genus of claimed compositions or claimed methods using the claimed compositions at the time of filing.

In response to the Examiner's rejection to claim 102, applicants note that this claim has been cancelled. Thus, the Examiner's rejection of claim 102 is now moot.

In response to the Examiner's rejection to claims 101, 108, 109 and 111-124, but without conceding the correctness thereof, applicants note that claims 101, 108, 109, 111-114, 117, 123 and 124 have been amended. These claims, as amended, do not recite the phrase "an altered ceramide portion comprising an altered sphingosine base." Instead, they only refer to an altered sphingosine base, the nature of the alteration being further defined elsewhere in the claim.

In view of the amendments to the claims, applicants maintain that claims 101, 108, 109 and 111-124 satisfy the requirements of 35 U.S.C. §112, first paragraph, and request that the Examiner reconsider and withdraw this ground of rejection.

Claim Rejection Under 35 U.S.C. §112, Second Paragraph - Indefiniteness

The Examiner rejected claims 101, 102, 108, 109 and 111-124 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant

regards as the invention. Specifically, the Examiner stated that claims 101, 113 and 114 are indefinite because the recitation "saponin derivable from the bark of a Quillaja saponaria Molina tree." The Examiner also stated that claims 102 and 111 are indefinite because of the recitation of "QS-21". The Examiner stated that the specification does not describe with sufficient clarity the chemical and structural nature of a "saponin derivable from the bark of a Quillaja saponaria Molina tree" or "QS-21." The Examiner stated that the specification appears to define the saponins by teaching that QS-21 is as an example of one of the saponins and to reference literature that teaches one how to isolate QS-21 from a mixture of saponins. The Examiner also stated that because the saponins or QS-21 appears to be an essential ingredient of the claimed invention, the attempt to describe QS-21 and how it is isolated is an attempt at incorporation by reference of matter essential to the practice of the claimed invention. The Examiner further stated that the references cited are not available for incorporation by reference because they are non-patent publications.

In response to the Examiner's rejection to claim 102, applicants note that this claim has been cancelled. Thus, the Examiner's rejection of claim 102 is now moot.

In response to the Examiner's rejection to claims 101, 108, 109 and 111-124, but without conceding the correctness thereof, applicants, as proposed by the Examiner in the attached February 6, 2004 Examiner's Proposed Amendments, have hereinabove amended the

specification to incorporate explicitly subject matter previously incorporated by reference from Kensil, et al. "Separation and Characterization of Saponins with Adjuvant Activity from *Quillaja saponaria* Molina Cortex", Journal of Immunology, 146(2):431-437 (January 15, 1991) and Newman, et al., "Saponin Adjuvant Induction of Ovalbumin-Specific CD8⁺ Cytotoxic T Lymphocyte Responses", Journal of Immunology, 148(8):2357-2362 (April 15, 1992). Kensil, et al. and Newman, et al. are expressly incorporated on page 66, line 10 and page 128, line 27 to page 129, line 2 of the specification, and are designated as reference numbers "10" and "11", respectively, in the Third Series of Experiments.

Specifically, applicants have amended the specification to incorporate the first two paragraphs of the "Materials And Methods" section on page 432 of Kensil, et al. and footnote 2 on page 2357 of Newman, et al.

In accordance with M.P.E.P. §608.01(p)(I)(A)(2), applicants' undersigned attorney states that the amendatory material from Kensil, et al. and Newman, et al. consists of the same material incorporated by reference in the referencing application, and that the specification, as amended, does not raise any issue of new matter.

In view of the above remarks, applicants maintain that amended claims 101, 108, 109 and 111-124 satisfy the requirements of 35 U.S.C. §112, second paragraph, and request that the Examiner reconsider and withdraw this ground of rejection.

The Examiner also rejected claim 111 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner stated that claim 111 is indefinite because the phrase "the saponin" lacks antecedent basis.

In response to the Examiner's rejection of claim 111, applicants note that claim 111 has been amended. Claim 111, as amended, does not recited the phrase "the saponin." Rather, amended claim 111 only refers to "QS-21."

In view of the above remarks, applicants maintain that amended claim 111 satisfies the requirements of 35 U.S.C. §112, second paragraph, and request that the Examiner reconsider and withdraw this ground of rejection.

Rejections Under 35 U.S.C. §103(a) - Obviousness

The Examiner rejected claims 101, 102, 113, 114 and 116-124 under 35 U.S.C. §103(a) as allegedly unpatentable over Wiegand (U.S. Patent No. 5,599,914, issued February 4, 1997) in view of Jennings (U.S. Patent No. 4,356,170, issued October 26, 1982), in view of Neurath (U.S. Patent No. 4,591,552, issued May 27, 1986), in view of Ratcliff (U.S. Patent No. 5,344,870, issued September 6, 1994), in view of Patrick (U.S. Patent No. 4,652,629, issued March 24, 1987), in view of Blincko (U.S. Patent No. 5,256,409, issued October 26, 1993), in view of Marciani (Vaccine,

9:89-96 (February 1991)), in view of Tsuchida (Journal of the National Cancer Institute, 78:45-54 (1987)), in view of Ritter (Seminars in Cancer Biology, 2:401-409 (1991)) and further in view of Livingston (Proc. Natl. Acad. Sci. USA, 84:2911-2915 (May 1987)).

The Examiner stated that Wiegand discloses glycoconjugates comprising gangliosides conjugated to carrier proteins, wherein the ganglioside has been ozonolyzed and reduced at the C-4 double bond of the sphingosine base to produce a reactive aldehyde intermediate that may be reacted directly with free amines present in carrier proteins to form a conjugate (citing to col. 1, line 11 to col. 2, line 44), wherein the ganglioside may be GM3, GD3, GM2 or GM1. The Examiner stated that Wiegand teaches that the coupling of gangliosides to carrier proteins is appropriate of all gangliosides, and that glycoconjugates of gangliosides are useful as vaccines (citing col. 1, lines 50-55). The Examiner acknowledged that Wiegand fails to explicitly teach that the bond between the aldehyde group of ozonolyzed and reduced ganglioside and the carrier protein would be via a lysine residue of the carrier protein. The Examiner also acknowledged that Wiegand fails to specifically teach a glycoconjugate comprising the specific carrier protein, KLH, and fails to teach a glycoconjugate having a ganglioside to KLH molar ratio of about 200:1 to 1400:1. The Examiner also acknowledged that Wiegand fails to teach a glycoconjugate within a composition containing a saponin. The Examiner also acknowledged that Wiegand fails to teach the specific range of amounts of conjugated ganglioside in a

composition, where the amounts are about 1µg to about 200µg.

The Examiner stated that Jennings discloses the chemistry of linking a carbohydrate containing a reactive aldehyde group to a carrier protein is well known and likely would be via a lysine (citing col. 3, lines 40-46 and claims 11 and 18 at cols. 9 and 10, respectively).

The Examiner stated that Ratcliff, Patrick and Blincko disclose that KLH was known as a useful carrier protein for carbohydrate antigens (citing Ratcliff, col. 29, lines 46-51), small peptide antigens (citing Patrick, col. 9, lines 7-28), and for tricyclic antidepressant drugs (citing Blincko, col. 7, lines 29-41).

The Examiner stated that Ritter discloses the desirability of conjugating gangliosides to KLH. The Examiner stated that Ritter discloses that covalent attachment to KLH results in the production of IgG antibodies in melanoma patients, and that the production of IgG antibodies is desirable because IgG antibodies are of higher affinity, better able to penetrate solid tissue, able to mediate antibody-dependent cell-mediated cytotoxicity and remains in the circulation for longer periods after immunization (citing page 106, 1st col.).

The Examiner stated that Neurath, using KLH as the carrier protein and the SPDP heterobifunctional linker method of Wiegand, teaches peptide-KLH conjugates contain approximately 200 peptide molecules per KLH (citing col. 17, lines 15-40).

The Examiner thus concluded that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention was made to have made the conjugates of the claimed composition, wherein the conjugates comprise a GM2 ganglioside covalently bound to via lysine residues of KLH, a well-known carrier protein, by reductive amination as taught by Jennings, and to have achieved a ganglioside-KLH molar ratio of between 200:1 to 1400:1, because Neurath teaches that a molar ratio of hapten to carrier protein of 200:1 can be achieved using KLH and using a method that attaches the hapten to KLH via lysine residues.

The Examiner stated that Wiegand fails teach a glycoconjugate within a composition containing a saponin. However, the Examiner stated that Marciani teaches that the use of 20µg of QS-21 as an adjuvant in a genetically-engineered subunit vaccine against feline leukemia virus, and teaches that the choice of QS-21 was important in achieving an immunogenic response to the recombinant viral peptide in that QS-21 was much more effective than alum or oil emulsions in eliciting a humoral response and were protected from viral challenges (citing page 94, col. 2, 2nd full paragraph to page 95, col. 1). The Examiner therefore concluded that it would have been *prima facie* obvious to one of ordinary skill in the art to have used an adjuvant such as QS-21 because QS-21 appears to be superior to other art known adjuvants such as alum and oil emulsions.

The Examiner stated that although Wiegand teaches a

glycoconjugate that comprises a GD3-lactone ganglioside, Wiegand fails to teach a glycoconjugate comprising GD3 lactone. However, the Examiner stated that Tsuchida teaches that GD3 is expressed in all of the human melanoma samples tested and Ritter teaches that a GD3 lactone (GD3 lactone I) induces antibodies that cross-react with GD3, and is the preferable antigen for use in a vaccine against melanoma cells that express GD3. The Examiner therefore concluded that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have used gangliosides other than that of GM2, such as GD3 lactone, because Tsuchida teaches that GD3 is a major ganglioside of melanoma cells and because Ritter teaches that GD3 lactone induces antibodies that cross-react with GD3.

The Examiner stated that Wiegand also fails to teach the specific range of amounts of conjugated ganglioside in a composition, where the amounts are about 1µg to about 200µg. However, the Examiner stated that Livingston teaches immunization of human melanoma patients with a dose of 100µg of an unconjugated GM2 ganglioside preparation (combined with BCG or S. Minnesota mutant R595) that produced an antibody response (citing page 2912, col. 2 to page 2913, and Table 2). The Examiner thus concluded that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have determined the appropriate amounts of KLH-conjugated ganglioside to administer.

The Examiner also stated that the claimed invention is also drawn to methods of treatment, either a method of

stimulating or enhancing production of an antibody to GD3 lactone or a method of treating a human subject having cancer comprising the administration of compositions comprising ganglioside conjugates. The Examiner stated that Wiegand suggests such methods because it teaches that ganglioside conjugates may be used as vaccines. The Examiner also stated that Livingston and Ritter both teach that melanoma patients respond to preparations comprising gangliosides and adjuvants by producing ganglioside and melanoma specific antibodies. The Examiner thus concludes that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention was made to use the ganglioside compositions comprising a conjugate of Wiegand where the carrier protein is KLH, as suggested by Ritter (and also Ratcliff, Patrick and Blincko) and further comprising an adjuvant such as QS-21 as taught by Marciani in methods of treatment for the production of antibodies to gangliosides, or for the treatment of a human subject having cancer. Optimization of the dosage, route of immunization, number of sites of immunization to administer the composition is well within the skill of the ordinary artisan.

In response to the Examiner's rejection to claim 102, applicants note that this claim has been cancelled. Thus, the Examiner's rejection of claim 102 is now moot.

In response to Examiner's rejection to claims 101, 113, 114 and 116-124, applicants respectfully traverse, and maintain that the Examiner has failed to establish a *prima facie* case of obviousness against the rejected

claims.

Briefly, claim 101, as amended, provide a composition which comprises (A) a conjugate of (i) a derivative of a GD3 lactone ganglioside which GD3 lactone ganglioside comprises an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and (ii) Keyhole Limpet Hemocyanin, wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin; (B) QS-21; and (C) a pharmaceutically acceptable carrier; wherein the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1 μ g and about 200 μ g, the amount of QS-21 is an amount of between about 10 μ g and about 200 μ g, the GD3 lactone:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and QS-21 is effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside. Claims 113, 114 and 116-124, as amended, provide for methods of stimulating or enhancing production of an antibody to the GD3 lactone ganglioside in a subject, and methods of treating a human subject having cancer, e.g., melanoma, by administering said composition to the subject.

The claimed invention is based on applicants' *surprising discovery* that a conjugate of a ganglioside derivative

covalently bound to Keyhole Limpet Hemocyanin, e.g., GM2-KLH, with QS-21 as an adjuvant, creates is a strikingly immunogenic vaccine, far superior to previous ganglioside-based vaccines, such as GM2 adherent to the surface of BCG, salmonella Minnesota mutant R595 or proteosomes, GM2-KLH only, and GM2-KLH plus DETOX or BCG, with regard to (1) higher IgM and IgG antibody titers against GM2 and (2) a decrease in systemic and local adverse reactions related to administering to a subject. See instant specification, page 58, line 24 to page 59, line 19, and page 93, line 16 to page 95, line 15.

To establish a *prima facie* case of obviousness, the Examiner must demonstrate three things with respect to each claim. First, the cited references, when combined, teach or suggest each element of the claim. Second, one of ordinary skill would have been motivated to combine the teachings of the cited references at the time of the invention. And third, there would have been a reasonable expectation that the claimed invention would succeed.

The references cited against the rejected claims fail to support a *prima facie* case of obviousness. Here, the cited references fail to support a *prima facie* case of obviousness. Specifically, to support a *prima facie* case of obviousness, one of ordinary skill would have to have been motivated to combine the teachings of the cited references at the time of the invention. Moreover, these references would also have to provide a reasonable expectation of success.

It is stressed that the Examiner has based this rejection

on the teachings of no fewer than ten references. Collectively, these references teach (1) a chemical modification of the sphingosine portion of glycosphingolipids and the subsequent coupling of such modified glycosphingolipids to other molecules, e.g., protein, (2) the preparation of antigenic polysaccharide-protein conjugates by directly conjugating a protein with an altered polysaccharide, (3) the use of KLH in synthesizing carbohydrate antigens, small peptide antigens and tricyclic antidepressant drugs, (4) covalent attachment of gangliosides to foreign carrier proteins, (5) a radioactive or enzyme labeled synthetic peptide conjugated to KLH which employs a *synthetic peptide:KLH* molar ratio of 200:1, (6) the use of 20µg of QS-21 as an adjuvant in a vaccine for cats against feline leukemia virus, (7) certain gangliosides, such as GM3, GD3, GM2 and GD2, are expressed in human melanoma specimens, and (8) the preparation of GM2-only, GM2/BCG or GM2/R595 vaccines using 100µg of purified, *unmodified* GM2 ganglioside. From these references, the Examiner draws the untenable conclusion that one of ordinary skill in the art would have been motivated to combine, and would have reasonably expected, this combination to work better than previous ganglioside-based compositions in treating cancer, such as melanoma.

Specifically, Wiegand teaches a chemical modification of the sphingoid portion of glycosphingolipids and the subsequent coupling of such modified glycosphingolipids to other molecules, such as proteins. Wiegand also discloses the preparation and subsequent coupling of reductively aminated ozonolysis products of the GM3, GD3,

GM2 and GM1 gangliosides. Wiegand, col. 5, line 24 to col. 16. Wiegand, however, does not teach or suggest any particular species of glycosphingolipid that would perform effectively as a vaccine when linked to other molecules to form an immunoconjugate composition, such as the claimed invention. Also, Wiegand neither teaches nor suggests that the modification and conjugation of a derivative of a particular ganglioside, such as GD3 lactone, would produce a composition having superior immunogenic properties relative to a composition obtained with the use of any other gangliosides that are listed in the reference.

Furthermore, as acknowledged by the Examiner herself in the outstanding Office Action, Wiegand fails to explicitly teach (1) the GD3 lactone ganglioside, (2) the bond between the aldehyde group of ozonolyzed and reduced ganglioside and a carrier protein would be via a lysine residue of the carrier protein, (3) a glycoconjugate comprising the specific carrier protein, KLH, (4) a glycoconjugate having a ganglioside to KLH molar ratio of about 200:1 to 1400:1, (5) a glycoconjugate within a composition containing a saponin, such as QS-21, and (6) the specific range of amounts of conjugated ganglioside in a composition, where the amounts are about 1µg to about 200µg.

Jennings teaches the preparation of antigenic polysaccharide-protein conjugates by altering a polysaccharide molecule via controlled oxidation and specifically coupling the altered polysaccharide with a free amino group of a protein via reductive amination.

Applicants respectfully disagree with the Examiner's contention that the conjugation procedure taught in Jennings, in combination with Wiegand, provides for the identical coupling procedures recited in the claimed invention. Specifically, applicants note that Jennings teaches *altering* the polysaccharide and *directly* conjugating the modified polysaccharide with a protein, e.g., tetanus toxoid TT, diphtheria toxoid and other proteins derived from bacteria, bovine serum albumin (BSA), or other proteins containing lysine residues such as a synthetic polylysine. Jennings, Abstract; col. 3, lines 3-54; col. 5, line 17 to col. 6, line 10. The claimed invention radically differs from Jennings in that (1) a ganglioside, e.g., GD3 lactone ganglioside, is used to conjugate with the carrier protein KLH, (2) the carbohydrate portion of the ganglioside is *unaltered* throughout the conjugation process, and (3) the conjugation between the ganglioside and the carrier protein KLH does not occur directly on the unaltered carbohydrate portion of the ganglioside, but rather, on the altered sphingosine portion of the altered ceramide portion of the ganglioside. Moreover, Jennings does not teach nor suggest KLH as a carrier protein. Thus, Jennings actually *teaches away* from the claimed invention by encouraging one skilled in the art to modify carbohydrates and directly conjugate carrier proteins to the terminal portion of the altered carbohydrate, and use carrier proteins other than KLH.

Ratcliff, Patrick and Blincko only teach that the carrier protein KLH may be useful in the synthesis of carbohydrate antigens, small peptides antigens and for

tricyclic antidepressant drugs, respectively. None of these references teach or suggest any gangliosides, such as GD3 lactone ganglioside, let alone specifically conjugating KLH with any gangliosides. Ratcliff merely provides a general list of carriers suitable for the synthesis of carbohydrate antigens. Such carriers "include proteins, such as the appropriate serum albumin, such as human or bovine serum albumin, keyhole limpet hemacyanin, tetanus toxoid, and the like." Ratcliff, col. 9, lines 1-4. Ratcliff does not teach or suggest the use of a particular carrier conjugated to a ganglioside that would produce a composition having superior immunogenic properties to treat cancer, such as melanoma.

Patrick discloses a laundry list of suitable carrier proteins for creating small peptides antigens. Patrick, col. 9, lines 7-28. Similar to Ratcliff, Patrick does not teach or suggest the use of a particular carrier, i.e., KLH, conjugated to a ganglioside that would produce a composition having superior immunogenic properties to treat cancer, such as melanoma.

Blincko provides a list of carrier proteins which include "keyhole limpet haemacyanin (KLH), bovine serum albumin (BSA), human serum albumin (HSA), polytufsin or other repeating unit polypeptides, polyamino acids or random copolymers of amino acids, or lysozyme or other enzymes." Blincko, col. 7, lines 29-37. Blincko discloses that KLH as the more preferred carrier protein since immunogens in which the carrier protein which comprises KLH are found particularly effective in raising high titre antisera.

Blincko, col. 7, lines 37-42. However, applicants stress that Blincko neither teaches nor suggests a ganglioside or the conjugation of KLH to a ganglioside.

Ritter teaches that the "covalent attachment of gangliosides to foreign carrier proteins such as KLH" can induce consistent IgG antibodies to gangliosides in the mouse. Ritter, page 406, col. 1. Ritter also teaches KLH-GM2 conjugates. However, Ritter does not describe the chemical nature of the conjugate or of how to make the conjugate. Hence, Ritter neither discloses anything conjugated through the ceramide portion of a ganglioside, nor enables making a conjugate where "the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and the nitrogen of the ϵ -aminolysyl group of Keyhole Limpet Hemocyanin." Furthermore, Ritter does not teach or suggest (1) an adjuvant such as QS-21, (2) the 200:1 to 1400:1 GD3 lactone:KLH molar ratio, or (3) the specified amounts of conjugated GD3 lactone ganglioside derivative recited in the rejected claims, as amended.

Neurath discloses a radioactive or enzyme labeled synthetic peptide of no more than 60 amino acids conjugated to KLH, which is employed as a diagnostic tool to determine the presence of Hepatitis B surface antigen. Neurath discloses a *synthetic peptide:KLH* molar ratio of 200:1. Nowhere does Neurath teach or suggest any gangliosides, particularly the GD3 lactone ganglioside, glycoconjugates or a ganglioside:KLH molar ratio. Furthermore, Neurath's specific teaching of a synthetic

peptide-KLH molar ratio of 200:1 does not teach the range of values, i.e., "from 200:1 to 1400:1" of ganglioside-KLH molar ratios as claimed in the instant invention.

Marciani teaches the use of 20µg of QS-21 as an adjuvant in a vaccine for cats against feline leukemia virus. Marciani also teaches that the vaccine consists of a recombinant protein, rgp70D, which is a non-glycosylated protein derived from the envelope glycoprotein of FeLV subgroup A envelope gene, that is absorbed on to aluminium hydroxide and used in conjunction with QS-21. Although Marciani teaches that "the purified saponin component elicited a high titre antibody response and also induced an affinity maturation of these antibodies," applicants strongly note that this observation is strictly limited to QS-21 when used in conjunction with a feline leukemia virus vaccine for cats. Nowhere would one skilled in the art associate this reference with the claimed invention. In other words, Marciani does not teach any gangliosides, glycoconjugates or the use of QS-21 with a glycoconjugate. Therefore, Marciani does not provide a motivation to combine such reference in combination with any of the cited references. To consider otherwise would be hindsight.

Tsuchida teaches certain gangliosides, such as GM3, GD3, GM2 and GD2, are expressed in human melanoma specimens. Nowhere in Tsuchida does it suggest cleaving the C4 position of the sphingosine base of any ganglioside to create a ganglioside derivative, and covalently binding such ganglioside derivative to Keyhole Limpet Hemocyanin. Nor does Tsuchida teach using such ganglioside derivative

with QS-21 as an adjuvant. Furthermore, Tsuchida does not teach any of the numerical values and ranges recited in the claimed invention.

The Examiner stated that Ritter teaches that a GD3 lactone induces antibodies that cross-react with GD3. Applicants respectfully note that the Examiner's above assertion of Ritter actually applies only to mice. Ritter, page 406, col. 2, 1st full para. Rather, Ritter teaches that "[i]n contrast to the mouse, however, the antibodies elicited by...GD3 lactones...in humans were specific for the respective immunogens, and showed no reactivity with GD3 in dot blot immune stains or immune thin-layer chromatography, and no reactivity with human melanoma cells expressing GD3." Id. Hence, Ritter does not provide a motivation or suggestion to combine the teachings of the cited references based on the cross-reactivity of GD3-lactone induced antibodies. Moreover, as stated above, Ritter neither discloses anything conjugated through the ceramide portion of a ganglioside, nor enables making a conjugate wherein the "GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and the nitrogen of the ϵ -aminolysyl group of Keyhole Limpet Hemocyanin." Furthermore, Ritter does not teach or suggest (1) an adjuvant such as QS-21, (2) the 200:1 to 1400:1 GD3 lactone:KLH molar ratio, or (3) the specified amounts of conjugated GD3 lactone ganglioside derivative recited in the rejected claims, as amended.

Livingston discloses vaccines containing either purified

GM2 only or purified GM2 with BCG or R595 as adjuvants. Livingston also discloses the preparation of such GM2 vaccines containing 100µg of purified, *unaltered* GM2 ganglioside. Livingston, page 2912, col. 2. Livingston radically departs from the claimed invention in that it neither teaches an altered ganglioside derivative that is *conjugated* to *KLH* nor the use of QS-21 as an adjuvant. Furthermore, the 100µg amount taught in Livingston applies only to unaltered GM2. Without a teaching to suggest otherwise, Livingston fails to teach the limitation of "the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1µg to 200µg" recited in claims 101, 111, 113 and 114, as amended.

According to the M.P.E.P. §2143.01,

"[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination."

In re Mills, 916 F.2d 680 (Fed. Cir. 1990) (emphasis added). As demonstrated above, there is simply no motivation or suggestion to combine the cited references to create the instant invention. The collection of cited references is the result of the Examiner's impermissible use of hindsight to combine these references based on knowledge of the applicants' invention and underlying discovery. None of the references cited by the Examiner give any suggestion, motivation or "indication of which

parameters [are] critical or [a] direction as to which of many possible choices is likely to be successful" to one skilled in the art to create (1) a composition which comprises (A) a conjugate of (i) a derivative of a GD3 lactone ganglioside which GD3 lactone ganglioside comprises an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and (ii) Keyhole Limpet Hemocyanin, wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin; (B) QS-21; and (C) a pharmaceutically acceptable carrier; wherein the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1 μ g and about 200 μ g, the amount of QS-21 is an amount of between about 10 μ g and about 200 μ g, the GD3 lactone:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and QS-21 is effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside, (2) methods of stimulating or enhancing production of an antibody to the GD3 lactone ganglioside in a subject by administering said composition to the subject, or (3) methods of treating a human subject having cancer, e.g., melanoma, by administering said composition to the subject. *In re O'Farrell*, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988). Essentially, one skilled in the art would have had to conduct undue experimentation to achieve

applicants' successful yet unexpected result.

Assuming for the sake of argument that the combination of Wiegand, Jennings, Neurath, Ratcliff, Patrick, Blincko, Marciani, Tsuchida, Ritter, and Livingston established a *prima facie* case of obviousness (which applicants vigorously dispute), applicants respectfully maintain that any such *prima facie* rejection would be rebutted by the fact that the claimed invention demonstrates an unexpected advantage, e.g., markedly superior immunogenic results when compared to previous ganglioside-based vaccines, such as GM2/BCG or GM2-KLH plus DETOX or BCG, with regard to (1) higher titers of IgM and IgG antibodies specific for GM2 even at lower doses, and (2) a decrease in systemic and local adverse reactions related to administering to a subject.

Applicants' specification teaches that QS-21, at any of the dosage used, resulted in a *qualitatively different* response than those achieved with the prior art adjuvants to GM2 ganglioside. Instant specification, page 93, line 16 to page 95, line 15. The immunogenic responses achieved with the use of GM2-KLH vaccines alone or with optimal doses of BCG or DETOX were *substantially less effective* than the claimed invention which includes QS-21. For example, even at the 10µg dose, all patients who were treated with the claimed composition produced IgG antibodies detectable by dot blot immune stains against GM2. On the other hand, with the same amount as above, patients who were treated with GM2-KLH alone or with optimal doses of BCG, salmonella Minnesota mutant R595 or proteosomes had only rarely resulted in more than 1

detectable IgG response per 6 immunized patients.
Instant specification, page 94, lines 20-27.

The instant specification also teaches that local reactions to dosages of 100-200µg of QS-21 were "quite different" in terms of local adverse reactions than those seen with comparable dosages of BCG and DETOX. Instant specification, page 94, line 5. It states that the local response is more diffuse than the response generally seen with doses of DETOX or BCG inducing comparable systemic symptoms. Instant specification, page 94, lines 8-11. It additionally teaches that a surprising feature of the subjects' response to QS-21 was that several days later (at most 10 days later) the local reactions had completely abated and there was no evidence that the vaccination had been administered to that site. Instant specification, page 97, Table 6. Furthermore, at the 100µg dose, patients treated with the claimed invention showed resulted in only 2 episodes of low grade fever in 44 injections and the local inflammatory responses, which were limited to 2-4 days, did not interfere with daily activities. Instant specification, page 93, lines 29-34.

Therefore, in view of the surprising nature of this invention, one of ordinary skill in the art would not have been able to predict, based on the cited references, whether the claimed invention would be *more effectively* immunogenic even at low dosages, and result in a decrease in systemic and local adverse reactions. Moreover, one of ordinary skill certainly would not have reasonably expected the superior effects over to previous ganglioside-based vaccines, such as GM2/BCG or GM2-KLH

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 43

plus DETOX or BCG as discussed above. To maintain otherwise would be hindsight.

In view of the above remarks, applicants maintain that claims 101, 113, 114 and 116-124, as amended, satisfy the requirements of 35 U.S.C. §103(a).

The Examiner also rejected claims 114 and 115 under 35 U.S.C. §103(a) as allegedly unpatentable over Wiegand (U.S. Patent No. 5,599,914, issued February 4, 1997) in view of Jennings (U.S. Patent No. 4,356,170, issued October 26, 1982), in view of Neurath (U.S. Patent No. 4,591,552, issued May 27, 1986), in view of Ratcliff (U.S. Patent No. 5,344,870, issued September 6, 1994), in view of Patrick (U.S. Patent No. 4,652,629, issued March 24, 1987), in view of Blincko (U.S. Patent No. 5,256,409, issued October 26, 1993), in view of Marciani (Vaccine, 9:89-96 (February 1991)), in view of Tsuchida (Journal of the National Cancer Institute, 78:45-54 (1987)), in view of Ritter (Seminars in Cancer Biology, 2:401-409 (1991)), in view of Livingston (Proc. Natl. Acad. Sci. USA, 84:2911-2915 (May 1987)), and further in view of Diatlovitskaia (Biokhimiia, 56(3):560-564 (1991); Abstract only).

The Examiner stated that claims 114 and 115 also read on methods of treatment of tumors of epithelial origin. The Examiner stated that the combination of Wiegand, Jennings, Neurath, Ratcliff, Patrick, Blincko, Marciani, Tsuchida, Ritter and Livingston fail to teach treating a cancer of epithelial origin. However, the Examiner stated that Diatlovitskaia teaches that the ganglioside

GD3 is overexpressed in breast carcinoma, which is an example of a cancer of epithelial origin. The Examiner thus concluded that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the ganglioside compositions comprising a conjugate of Wiegand where the ganglioside was a GD3 lactone, where the carrier protein is KLH, as suggested by Ritter (and also Ratcliff, Patrick and Blincko) and further comprising an adjuvant such as QS-21 as taught by Marciani in methods for the treatment of a human subject having an epithelial cancer.

In response to the Examiner's rejection of claims 114 and 115, applicants respectfully traverse this rejection for the reasons provided below.

Briefly, claims 114 and 115, as amended, provide for methods of treating a human subject having cancer which comprises administering to the subject an effective amount of a composition which comprises (A) a conjugate of (i) a derivative of a GD3 lactone ganglioside which GD3 lactone ganglioside comprises an unaltered sphingosine base, wherein the derivative differs from the GM2 ganglioside solely by having an altered sphingosine base which retains only C1 through C4 from the unaltered sphingosine base of the GD3 lactone ganglioside, and (ii) Keyhole Limpet Hemocyanin, wherein the GD3 lactone ganglioside derivative is covalently bound to Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base and a nitrogen of an ϵ -aminolysyl group of Keyhole Limpet Hemocyanin; (B) QS-21; and (C) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GD3 lactone ganglioside derivative is an amount between about 1 μ g and about 200 μ g, the amount of QS-21 is an amount of between about 10 μ g and about 200 μ g, the GD3 lactone:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and QS-21 is being effective to stimulate or enhance production in a subject of an antibody to the GD3 lactone ganglioside, and thereby treat the cancer. In one embodiment, the cancer is of epithelial origin.

The references cited against claims 114 and 115 also fail to support a *prima facie* case of obviousness.

It is stressed that the Examiner has based this rejection on the teachings of no fewer than eleven references. Wiegand, Jennings, Neurath, Ratcliff, Patrick, Blincko, Marciani, Tsuchida, Ritter and Livingston have been discussed above.

Diatlovitskaia teaches that the GM3, GD3, GM1 and GM4 gangliosides are found on or in human gastric and mammary tumors. Diatlovitskaia, however, does not provide the elements missing from the references discussed above, i.e., it does not disclose or suggest the claimed composition comprising a conjugate covalently bound as recited in the claims, and also including QS-21, or a method of using such composition to enhance or stimulate antibody production, to treat cancer, or to prevent relapse of melanoma in patients at risk of such relapse, or the numerical values recited in the claimed invention.

Furthermore, as taught by Ritter, GD3 lactone-induced antibodies do not cross-react with GD3 in human subjects. Ritter, page 406, col. 2, 1st full para. Rather, Ritter teaches that "[i]n contrast to the mouse, however, the antibodies elicited by...GD3 lactones...in humans were specific for the respective immunogens, and showed no reactivity with GD3 in dot blot immune stains or immune thin-layer chromatography, and no reactivity with human melanoma cells expressing GD3." Id. Hence, Ritter does not provide a motivation or suggestion to combine the teachings of the cited references based on the cross-reactivity of GD3-lactone induced antibodies.

For these reasons, claims 114 and 115, as amended, are patentably distinct over Diatlovitskaia in combination of Wiegand, Jennings, Neurath, Ratcliff, Patrick, Blincko, Marciani, Tsuchida, Ritter and Livingston.

Moreover, as discussed above, the claimed invention demonstrates an unexpected advantage, e.g., markedly superior immunogenic results to previous ganglioside-based vaccines, such as GM2/BCG or GM2-KLH plus DETOX or BCG, with regard to (1) higher titers of IgM and IgG antibodies specific for GM2 even at lower doses, and (2) a decrease in systemic and local adverse reactions related to administering to a subject.

In view of the above remarks, applicants maintain that claims 114 and 115, as amended, satisfy the requirements of 35 U.S.C. §103(a).

Applicants: Philip O. Livingston and Friedhelm Helling
Serial No.: 08/475,784
Filed: June 7, 1995
Page 47

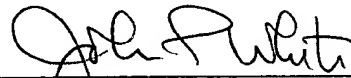
Summary

Applicants maintain that claims 101, 108, 109 and 111-124, as amended, herein are now in condition for allowance. Accordingly, a notice of allowance is respectfully requested.

If a telephone conference would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed \$490.00 fee for a three-month extension of time, is deemed necessary in connection with the filing of this Amendment. However, if an additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

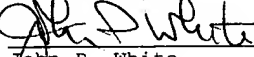
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